Relationship between phenol degradation efficiency and microbial community structure in an Anaerobic SBR

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Abstract
Phenol is present in wastewaters from various industrial processes, including petrochemical refineries, coke production and chemical compounds production such as herbicides, pesticides, insecticides, antioxidants and paper additives, among others. In this study, the efficiency of an Anaerobic Sequencing Batch Reactor (ASBR) fed with increasing phenol concentrations (from 120 to 1200 $mg \cdot L^{-1}$) was assessed and the relationship between phenol degradation capacity and the microbial community structure was evaluated. Degradation rates of phenol in ASBR ranged from 317 to 129 $mg \cdot L^{-1} \cdot d^{-1}$ at initial in-reactor phenol concentrations of 120 and 1200 $mg \cdot L^{-1}$, respectively. Phenol degradation rates achieved a quite constant value of about 330 $mg \cdot L^{-1} \cdot d\text{-}^{1}$ (a specific degradation rate of about 27 $mg \cdot VSS^{-1} \cdot d^{-1}$) for phenol concentrations above 800 $mg \cdot L^{-1}$. Operation at highest concentrations resulted in an efficient but slower process (about 130 $mg \cdot L^{-1} \cdot d^{-1}$, with specific degradation rate of 11 $mg \cdot VSS^{-1} \cdot d^{-1}$).Microbial diversity evolved from the inoculum to a quite stable structure for moderate phenol concentrations. However, for the highest value a drastic modification of the structure was observed. This indicating a quite direct relationship between the reactor performance and the microbial community.

Keywords: Anaerobic Digestion; ASBR reactor; Phenol; Microbial Communities.

INTRODUCTION

Phenol is an important chemical compound whit a number of applications and its global production is estimated to reach 6 million tons per year (Busca *et al*., 20008). Phenol is present in wastewaters from various industrial processes including petrochemical refineries, coke production and chemical compounds production such as herbicides, pesticides, insecticides, antioxidants and paper additives, among others. Concentration of phenol in wastewaters depends on the industrial activities, from 6-500 $mg \cdot L^{-1}$ in petroleum refinery, to 28-3900 $mg \cdot L^{-1}$ in coke and 2.8-1220 $mg \cdot L^{-1}$ in petrochemical wastewaters (Levén *et al*., 2012).

The presence of phenol can induce adverse effects in wastewater treatment processes, particularly in biological processes, due to its inhibitory effects on the activity of microorganisms responsible for the degradation of organic matter. The presence of phenol usually results in a decrease of the elimination rate and in longer treatment times required. Different anaerobic technologies have been implemented to cope with the presence of phenol because of the advantages they offer over other biological unit operations: high organic loading rates and low sludge production, in addition to energy production. Among them, Anaerobic Sequencing Batch Reactors (ASBR), given their various advantages such as operational simplicity, efficient quality control of the effluent, flexibility of use and high biogas yield (Tauseef *et al.*, 2013) can counteract the inhibitory effects of phenol and favor its degradation (Donoso-Bravo *et al*., 2009).

The aim of this paper is to study the efficiency of an Anaerobic Sequencing Batch Reactor (ASBR)

fed with increasing phenol concentrations (from 120 to 1200 $mg \cdot L^{-1}$) and to assess the relationship between phenol degradation capacity and the microbial community structure.

MATERIALS AND METHODS

BMP (Biochemical Methane Potential) test

Glass bottles of 400-500 mL-effective volume were used. The initial biomass concentration was 1.5 g $VSS \cdot L^{-1}$.Triplicate assays were carried out with two different types of carbon sources: only phenol, or phenol + glucose. The initial phenol and glucose concentration were 200 ± 8.5 $mg \cdot L^{-1}$ and 1.5±0.5 $g \cdot L^{-1}$, respectively. The biogas production and composition were measured daily during the first six days, and then every 2-3 days. Liquid samples were taken weekly for determination of the soluble chemical oxygen demand (CODs) and phenol and volatile fatty acids (VFA) concentrations.

Two different inoculum sludge were used, coming from either a brewery WWTP (Wastewater treatment plant) or a tobacco processing WWTP. The initial methanogenic activity of inocula was 0.2-0.3 and 0.15-0.2 g $COD_{CH4} \cdot m^{-3} \cdot d^{-1}$ for brewery and tobacco processing plant, respectively. After depletion of phenol, the medium was supplemented once with new addition of phenol at 200 $mg \cdot L^{-1}$.

ASBR reactor

The reactor has an effective liquid and headspace volumes of 5 L and 1 L, respectively. The ASBR was operated in cycles as follows: filling (20 min), reaction (variable duration enabling 90% of phenol degradation), settling (1h) and discharge (20 min). Liquid recirculation was used for reactor mixing. The reactor was fed with synthetic wastewater containing phenol at different concentrations (Table 1) and sodium bicarbonate to maintain alkalinity in a range 2.5-3.0 g $CaCO3 \cdot L^{-1}$.

Sludge from a tobacco processing WWTP was used as inocula. The biomass concentration in the ASBR reactor was kept constant during all the experiments at 12 g $VSS \cdot L^{-1}$.Three different phases can be considered: (a) start up, (b) acclimation to phenol, and (c) testing period under increasing phenol concentrations (Table 1).

Table 1. Substrate concentration and operation time of ASBR reactor.

Period	Operation time (d)	Number of cycles
Start up (Glucose 5 g $COD \cdot L^{-1}$)	0-21 (21 days)	15-20
Acclimation (Phenol 120-240 $mg \cdot L^{-1}$)	0-80 (80 days)	20
I (Phenol 120 ± 60 $mg \cdot L^{-1}$)	81-120 (39 days)	22
II (Phenol 240 ± 4 $mg \cdot L^{-1}$)	121-139 (18 days)	7
III (Phenol 500 ± 50 $mg \cdot L^{-1}$)	140-161 (21 days)	6
IV(Phenol 800 ± 7 $mg \cdot L^{-1}$)	162-195 (33 days)	7
V (Phenol 1200 ± 43 $mg \cdot L^{-1}$)	196-281 (85 days)	4

For microbial community characterization, samples were taken from reactor at the end of the last cycle of operation under each phenol concentration tested.

Analytical Methods

CODs and alkalinity (total and partial) were measured following Standard Methods (APHA, 1995). VFAs and phenol were detected and quantified by a gas chromatograph GC-8A (Shimadzu Japan).
DNA was extracted using Powersoil DNA Isolation Kit, MO BIO Laboratories Inc (Carlsbad, CA, US). PCR amplification was carried out a MJ-Mini thermocycler (Bio-Rad Laboratories Inc, Hercules, CA, US) with primers targeting both the V3 region of 16S rDNA (total community) and a functional gene involved in phenol degradation. PCR products were separated by Denaturing Gradient Gel Electrophoresis (DGGE) using the DCode System (Bio-Rad Laboratories Inc, Hercules, CA, US). Gel photograph was analyzed with Bionumerics software to asses community

diversity and dynamics (Applied Maths, Sint-Martens-Latem, Belgium) and bands of interest were sequenced (Macrogen, Korea).

RESULTS AND DISCUSSION

BMP test

A lag time of 20-25 days was observed after the first addition of phenol, and the degradation was completed after 40 to 45 days. After the second addition, lag time decreased to 10 days and degradation was completed in 20-25 days, thus revealing that biomass did adapt to phenol showing a faster consumption response. After the third phenol addition, lag and total times were 4 and 11 days, respectively.

A similar degradation trend was obtained for brewery inoculum, with very similar lag time, and similar adaptation to repeated substrate additions (Table 2). Both inocula enabled the complete phenol degradation, although with different maximum and specific degradation rates (Table 2). As the inoculum from tobacco factory WWTP displayed a higher activity, it was selected for the following experiments.

Table 2. Lag and total time, maximum volumetric and specific degradation rate in batch assays.

		1st	2nd	3rd
Lag time/total degradation time (d)	Brewery	22/42	10/22	ND
	Tobacco	25/40	10/20	4/11
Maximum degradation rate (mg phenol·L^{-1}·d^{-1})	Brewery	6.3±0.2	7.6±0.4	ND
	Tobacco	10.8±0.3	13.5±0.3	35.5±0.5
Specific degradation rate (mg phenol·g VSS^{-1}·d^{-1})	Brewery	4.2±0.2	5.1±0.4	ND
	Tobacco	7.2±0.3	9.0±0.3	23.7±0.5

ASBR reactor assays

During the start-up phase, glucose degradation time was less than 24 h (data not shown). Acclimation to phenol was carried out during 20 cycles with a total duration of 80 days. In the last cycle, phenol (120 mg·L^{-1}) was completely depleted in 24 h, indicating a high capacity of the biomass to cope with phenol as the sole carbon source.

***Degradation kinetics at different phenol concentrations*:** Anaerobic biomass required approximately 4-6 cycles of operation to adapt to each phenol concentration, as observed by the decrease of the reaction time during the first cycles (Figure 1). Afterwards, the reaction time remained quite constant during the following cycles at each inlet concentration. Moreover, increasing phenol concentration resulted in a lengthening of the reaction time necessary to degrade at least 90% of the inlet phenol. For phenol concentration above 800 mg·L^{-1}, the reaction time increased exponentially with inlet concentration, probably due to an inhibitory effect, which required to increase the reaction period and operate the ASBR at lower OLR.

Microbial community investigation: In the ASBR under study, the community structure revealed by DGGE was dynamic with time and clustered according to time and inlet phenol concentration. The increase of phenol concentration was accompanied by a decrease of microbial diversity, suggesting the specialization of the community through the emergence and selection of the most adapted phylotypes, which may probably play a significant role in the maintenance of high degradation capacity. CCA results (Figure 2), shown that the samples corresponding to low and intermediate phenol concentrations (120 to 800 mg·L^{-1}) were closely grouped together. Therefore it seems that the influence of phenol concentration on the community structure was level-dependent: the increase of phenol concentration from 120 to 800 mg·L^{-1} had no significant effect on the community structure, while it involved drastic structural changes when increasing from 800 to 1200 mg·L^{-1}.

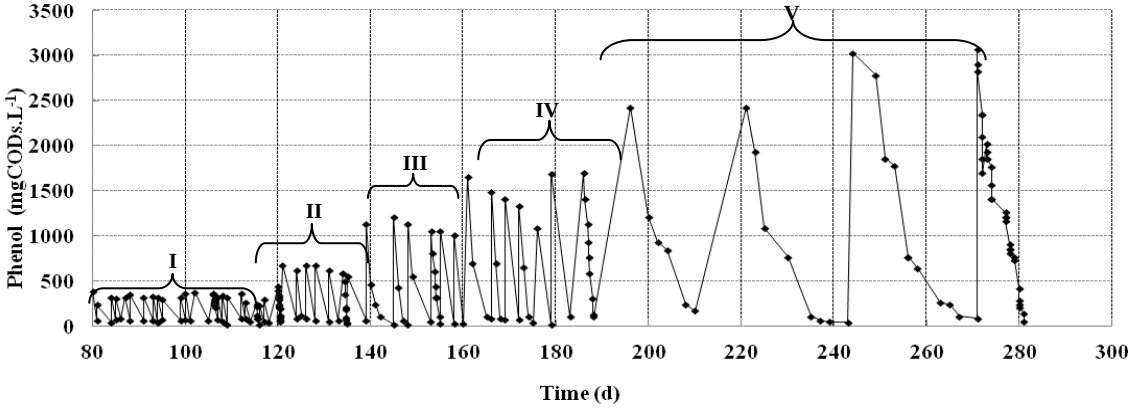

Figure 1. Phenol concentration (in COD basis) in consecutive ASBR cycles at different feeding concentrations (120, 240, 500, 800 and 1200 mg phenol·L^{-1}).

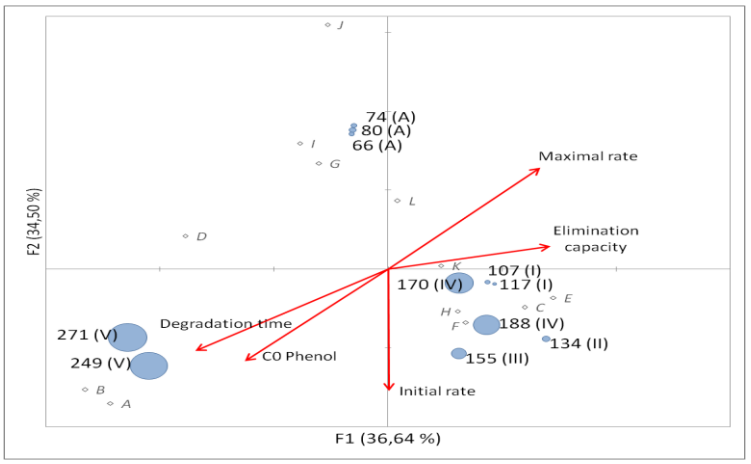

Figure 2: Canonical Correspondence Analysis (CCA) of microbial community patterns generated by 16S-DGGE analysis of 11 ASBR community samples (circles). The size of the circles was proportional to the inlet phenol concentration. The numbers indicate the sampling day, and the testing phase is indicated in parenthesis. The selected environmental variables (arrows) explained 66% of the inertia of the DGGE data set. Among all the individual variables (DGGE bands) only the excised and sequenced ones are

CONCLUSION

ASBR appears to be a good technological option for phenol degradation. The high removal performance despite the high phenol concentration may be a result of the strategy of reactor operation, based on the progressive increase of inlet phenol concentration, allowing for an enhanced biomass acclimation. However, it appears that working at concentrations above 1 g·L^{-1} (reported as inhibitory), result in a lost of operation efficiency. Results at microbiological level show that the increase of phenol concentration was accompanied by a decrease of microbial diversity and a progressive selection of the most adapted phylotypes.

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